ERN MEDICINE, and glance over the very large list of proposed laws which have relation to the interests of Public Health and Medical Practice.

A rapid survey of the long list there given, cannot do other than impress one with the heavy tasks which confront the C.M.A. Committee on Public Policy and Legislation in its work. (Doctor Dwight H. Murray, of Napa, Chairman.)

Physicians have cause to be grateful that the medical profession contains colleagues who are willing to interfere with the routine of their own busy, professional lives, and give wholehearted service to conserve and promote the best interests of scientific and organized medicine.

Fortunately, this 55th California Legislature will not be called upon to consider, as a "must-pass measure," a compulsory health insurance act. That does not mean, however, that the proponents of such legislation have given up the battle. On the contrary, it may be safely assumed that they are quietly marshalling their forces, to attain the same objectives, without resort to open battle. Wherefore, it behooves the medical profession to be as much on the alert, as in the past.

EDITORIAL COMMENT†

COMPLEXITY OF SERUM COMPLEMENT

The time is rapidly approaching when physicians and laboratory technicians must familiarize themselves with the clinical implications of the newer knowledge of the chemical nature of "alexin" or "serum complement." A typical example of recent advances is contained in a report by Pillemer[1] and his coworkers of the Institute of Pathology, Western Reserve University, of the four complementogenic factors active in specific serum hemolysis.

About fifty years ago it was shown by Buchanan, Bordet and others that cytolytic serums are inactivated if heated to 56° C for 30 minutes, and that such heated serums can be reactivated by the addition of normal serum. The nonspecific thermolabile factor or group of factors in normal serum bringing about this reactivation was named "alexin" by Bordet, and "serum complement" by Ehrlich. This thermolabile component or group of components was pictured by both investigators as a pan-immunizing defensive enzyme, causing lysis of bacteria or erythrocytes "sensitized" by the thermostable specific immune body ("amboceptor"). Complement, therefore, was assumed to be a single chemical substance, presumably protein in nature. This assumption has determined the terminology and clinical logic for the last half century.

The first serious challenge to this unitarian concept was about thirty years ago, when it was shown by a number of serologists that complement can be separated into two components, a globulin and an albumin fraction. Neither of these substances is in itself active, but full activity can be restored by reuniting these two proteins in their original proportions. Alexin or complement thus became a complex globulin-albumin conjugate, a globulin fraction ("mid-piece") capable of uniting directly with sensitized cells, to which was attached an albumin fraction ("end-piece"), incapable of direct union. Both "mid-piece" and "end-piece" were apparently denatured by heat.

This concept of complement as two conjugated proteins had little or no effect on clinical logic. A third essential complementogenic factor, however, was subsequently demonstrated by Coca,[2] who found that complement can be inactivated by adsorption or absorption on yeast, without injury to either the mid-piece or end-piece. After yeast inactivation the serum can be fully reactivated by addition of the thermostable fraction of normal serum. Ten years later a fourth essential complementogenic factor was demonstrated by Gordon,[3] a heat-stable component removed, destroyed or denatured by treatment with ammonia or ammonium salts.

Numerous possible clinical applications of the new complement components were implied in studies of the "hereditary absence of complement" in certain strains of guinea pigs. These strains are hyper-susceptible to practically all microbic infections, and can be reared only with most scrupulous hygienic care. It was shown by Hyde[4] that this hereditary serum deficiency is due solely to a lack of the third or yeast-adsorbed complement component, the other complematogenic factors being both qualitatively and quantitatively normal. Incidentally, Hyde showed that this third component does not pass through the placenta, that its lack of formation is due to a single recessive gene, and that failure to allow for the third component in heat-inactivated sera may introduce serious errors in routine clinical diagnosis.

Since then numerous biochemists have attempted to determine the method of action of each of the four recognized complementogenic components. A simplified terminology was suggested by Pillemer, Heidelberger[5] and others: C'1, C'2, C'3, and C'4 in place of the "mid-piece," "end-piece," "third component," and "fourth component" of earlier investigators. Pillemer and his colleagues found no evidence that any one of these components is able to unite directly with specific antibody ("amboceptor"), nor with non-sensitized cells. After "amboceptor" enters into primary combination with the red cells, however, the surface of the resulting antiserum-cell aggregate has a selective affinity for C'1, C'2 and C'4. C'1 is able to combine directly with these sensitized cells, while C'4 does not combine in the absence of C'1. C'1, therefore, apparently functions as a secondary sensitizer. Although C'1 is essential for C'4 absorption, an excess of C'1 may block absorption and thus function as an anti-complement.

The latest electrophoretic diagrams[6] suggest that C'1 and C'2 each consists of a mixture of four or more proteins, the essential fraction in each complex being still undetermined. After

adsorption of C'1, C'2 and C'4 the sensitized cells become susceptible to C'3, which is apparently a catalyst, not fixed by the cells and not used up in the process of hemolysis.

These results are typical of conclusions previously drawn by the same authors from a study of complement fixation[6] and specific precipitin reactions.[7] The tentative suggestion that at least one of the newly recognized complement components may be a derivative of certain vitamins, or is in some unknown way linked with sex-hormones, is perhaps prophetic of futural clinical implications of the newer knowledge. The relation of the newer complexities to problems of blood or plasma transfusion has not yet been adequately studied.

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A NEW TRANSFUSION HAZARD?

While making serological studies of transplanted rabbit cancers, Kidd and Friedewald[1] of the Rockefeller Institute noted that the serum of a normal rabbit often fixes complement in mixtures with saline extracts of its own tissue. They conclude from this that there is a natural antigen-antibody relationship between serum and certain fixed tissue components, somewhat analogous to the relationship now recognized between type specific hemagglutinins and red blood cells.

The test antigens used by the Rockefeller Institute immunologists were made from normal rabbit tissues. These were removed with aseptic precautions and used either fresh or after preservation in the frozen state (-22°C). The tissues were first ground with sand and the resulting paste suspended in a measured volume of physiological salt solution. The suspensions were afterwards freed from cellular débris by 20 minutes' low-speed centrifugation (4400 r.p.m.). The resulting slightly to moderately opalescent supernatant fluids varied in their complement-fixing titers according to their tissues of origin. Kidney extracts for example usually gave complement fixation (++++ reactions) when tested in dilutions as high as 1:320. Lung extracts gave similar reactions only in dilutions as high as 1:80, splenic extracts at 1:40, and heart muscle at 1:20, extracts of skeletal muscles being wholly inactive.

The initial opalescent extracts were subject for one hour to high-speed centrifugation (25,000 r.p.m.) and the resulting ultrasediment resuspended in phosphate buffer solution. This resuspension contained all of the reacting substances of the original crude extract, practically no reacting material remaining in the clear supernatant fluid. Kidd and his associates did not attempt to determine the nature of this ultrasediment, though Henle and Chambers[2] had previously shown by microscopical and tinctorial methods that such ultrasediments are presumably composed mainly of mitochondria. If we accept this conclusion, the complement-fixing property of normal rabbit serum would be pictured as due to an anti-mitochondrial antibody normally present in rabbit serum. The fact that the active component resists heating to 56°C, but is destroyed at 65°C, and that it can be salted out with ammonium sulfate confirms its antibody nature.

Kidd and Friedewald concerned themselves mainly in devising methods to prevent interference of their new complement-fixing antibody in routine virus and cancer research. Nevertheless many of their data are of suggestive clinical interest. This is particularly true of the absence of the new anti-mitochondrial antibody in the serum of rabbits less than one month old, suggesting that the new antibody may possibly function as an adult growth inhibitor. Marked difference in titer were noted in different strains or breeds of rabbits, one rabbit giving a ++++ complement-fixation reaction when its serum is tested in dilutions as high as 1:32, while other rabbits require a concentration as high as 1:2 for a similar reaction. Many serums are wholly inactive. This suggests the possibility that a high-titer pregnant mother might inhibit or modify the growth of its carried embryo by transplacental passage of the new antibody, a phenomenon somewhat analogous to that now recognized for the anti-Rh factor. Of even more interest is the suggested possibility of a new transfusion toxicity, particularly in the use of high-titer donors and low-titer or negative recipients. While acute anaphylactic shock would presumably not take place due to the intracellular location of the reacting antigen, other less dramatic reactions are by no means ruled out. Such speculative hazards will undoubtedly be the subject of future experimental study.

Meanwhile, the discovery of a new, and previously unsuspected natural antibody reacting specifically with fixed tissue intracellular granules (presumably mitochondria), is of wide biological interest, particularly since the kidney, liver, brain and testicle are among the organs most highly susceptible to this new serum factor. A basic discovery of this type may well initiate a new era in clinical theory and practice, similar to that initiated by Landsteiner's demonstration of hereditary blood groups.

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For References to Article, "A New Transfusion Hazard," see page 227.